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Nonmutagenic Carcinogens Induce Intrachromosomal Recombination in Dividing Yeast Cells

by Robert H. Schiestl

A large number of animal and human carcinogens without apparent genotoxic activity exist (nonmutagenic carcinogens) that are difficult or impossible to detect with the currently used short-term tests. Because of the association of carcinogenesis with genome rearrangement, a system selecting for intrachromosomal recombination (DEL recombination) that results in genome rearrangement has been constructed in the yeast *Saccharomyces cerevisiae*. Because DEL recombination is under different genetic control than interchromosomal recombination and meiotic recombination, it is probably due to a different mechanism. It has been found that DEL recombination is readily inducible by 10 mutagenic carcinogens and 17 nonmutagenic carcinogens that are not detectable (false negatives) with the Ames assay. In addition, three out of four mutagens that do not cause cancer (false positives in the Ames assay) do not induce DEL recombination. DEL recombination is inducible by UV only in dividing cells but not in cells synchronized in the G_1 or G_2 phase of the cell cycle. Interchromosomal recombination, on the other hand, is inducible in G_1 but not in G_2. The nonmutagenic carcinogens induce DEL recombination only in actively growing cells, which may give some indication as to their mechanism. Further characterization of the mechanism involved in induction of DEL recombination may contribute to the understanding of the biological activity of nonmutagenic carcinogens.

Introduction

It has been estimated that about 70–90% of new cases of cancer are linked to environmental carcinogens. A majority of these new cases might be preventable if the main risk and antirisk factors could be identified (*1*). At present, the regulation of environmental carcinogens is primarily based on long-term animal bioassays. Long-term animal bioassays are very costly and take 2–4 years to perform. Short-term bioassays, on the other hand, are very inexpensive by comparison, and they may produce results within 1 week or sooner. Therefore, prescreening chemicals with a short-term test would be highly desirable, especially if it would be possible to predict the carcinogenic potential of any given substance with a reasonably high level of accuracy (*2*). This would be especially important for industries producing pharmaceuticals, cosmetics, and food additives, because potential carcinogenic effects could be identified at an early stage in the development of a product.

A large amount of data establishes that animal and human carcinogens exist that do not exhibit any apparent genotoxic activity. These carcinogens are difficult or impossible to detect by the currently used short-term tests (*3*). The Ames or Salmonella assay, which is based on the induction of mutations in *Salmonella typhimurium* (*4*), and which is currently the most widely used short-term test, has recently been reported to detect only 56% of carcinogens (*5*). There are more than 60,000 synthetic chemicals in commercial use. New chemicals are introduced at a rate of 1,000 per year, some of which may pose a significant health risk to humans. The overwhelming majority of chemicals in our environment have not been tested for their carcinogenic activity in long-term studies. More chemicals have been tested with short-term tests, but there is considerable concern that there may be a large number of carcinogens in our environment that cannot be detected by conventional short-term tests. Long-term tests are not inherently suitable for solving this problem in the near future. On the other hand, short-term

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studies, if their sensitivity could be increased, might be used to solve this problem.

Because animal carcinogens exist that do not exhibit any apparent genotoxic activity, the scientific community has tried to explain the carcinogenic effect of these chemicals in many different epigenetic ways (*6*). Also, because models for carcinogenesis of nongenotoxic carcinogens are largely missing, a significant regulatory dilemma exists in assessing their risk to humans (*7*).

Another problem with long-term bioassays that use whole vertebrate animals is the growing public concern about the suffering of laboratory animals. The pressure from animal-welfare advocates, the high cost, and the delays imposed by animal testing for bringing chemicals to the marketplace are pushing regulatory agencies to decrease the number of animal tests required. The ultimate solution to this dilemma would be *in vitro* tests that can partly replace or limit the number of the whole-animal tests. Such an *in vitro* test is presented in this paper.

The first study that established an association between cancer-causing activity and the mutagenic activity of many different agents was carried out by Ames and co-workers (*4*). In this study, a 90% correlation between carcinogenicity and mutagenicity was reported for 300 chemicals. Since then, the Ames assay or Salmonella assay, based on the induction of mutations in *Salmonella typhimurium*, has been widely used and is currently the best characterized short-term test. However, in recent reports, only about 56% of the carcinogens tested have been reported to be detectable with the Salmonella assay (*5*). In an international collaborative study with 42 coded chemicals evaluating 30 different short-term tests in more than 50 different laboratories (*8*), it was found that the best assays identified only 70% of the chemicals correctly and that two-thirds of all tests identified only between 40 and 60% of the chemicals correctly. Sixty laboratories have taken part in a second similar study with eight carcinogens and two noncarcinogens (*9*). The overall performance was between 40 and 60% of the chemicals classified correctly. Another study (*10*) compared 4 *in vitro* tests with 73 chemicals which were sufficiently tested for their carcinogenic activity by the National Toxicology Program (NTP). These assays identified between 60 and 62% of the chemicals correctly. Furthermore, it has recently been reported by Ashby and Tennant (*5*) that the Salmonella assay identified only 63% of 301 chemicals correctly. These chemicals were sufficiently tested for their carcinogenic activity in long-term bioassays by the U.S. NTP. Furthermore, 65% of all chemicals were correctly identified on the basis of structural alerts. These results led Ashby and Tennant (*5*) to the conclusion: "Structural alerts and mutagenicity to Salmonella are useful but nondefinitive indicators of the overt carcinogens in the database, and the activity of the remaining (putative nongenotoxic) carcinogens is not predictable using current techniques.

Materials and Methods

Strains and Media

The diploid strain RS112 (*MATa/α ura3-52/ura3-52 leu2-3,112/leu2-Δ98 trp5-27/TRP5 arg4-3/ARG4 ade2-40/ade2-101 ilv1-92/ILV1 HIS3::pRS6/his3-Δ200 LYS2/lys2-801*) (*11*) was used and contains the deletion (DEL) system on one homolog (*HIS3*::pRS6) and a deletion of the entire open-reading frame of *HIS3* on the other homolog (*his3-Δ200*, 12). YPAD (yeast extract 1%, peptone 2%, dextrose 2%, adenine sulfate 30mg/L, agar 2% in distilled water) and synthetic minimal media (SC) were prepared as described previously (*13*).

Determination of the DEL Recombination Rates and Interchromosomal Recombination Frequencies

Plasmid pRS6 containing an internal fragment of the *HIS3* gene has been integrated at the genomic *HIS3* site. This resulted in two copies of the *his3* gene, one with a terminal deletion of the 3' end and the other with a terminal deletion at the 5' end [Fig. 1 (*14*)].

In cells of strain RS112 carrying the genomic integration of the plasmid pRS6, about 99% of *HIS3*$^+$ recombinants lose the *LEU2* gene [Fig. 1 (*14*)]. Therefore, the cultures used to select for HIS3$^+$ recombinants were pregrown on medium lacking leucine, and after treatment they were plated onto medium lacking histidine. Thus, growth and accumulation of recombinants does not occur in the preculture, and therefore the *HIS3*$^+$ frequency is a measure of the recombination rate which results in high reproducibility of spontaneous rates. Strain RS112 is also heteroallelic for *ade2-40* and *ade2-101* so that interchromosomal recombination (ICR) between homologs can be measured.

The influence of various agents on the frequency of deletions and interchromosomal recombination was determined as described (*15*). Single colonies were picked from YPAD medium and were inoculated into 5–25 mL of SC-LEU medium and grown for 24 hr at 30°C under constant shaking. Cells were counted, and the cell density was adjusted to 2×10^6 cells/mL in fresh SC-LEU medium. The medium containing the cells was distributed in aliquots of 5 mL each in disposable 15 mL tubes. The agent to be tested was added, the tubes sealed, and the cells were incubated for 17 hr at 30°C under constant shaking. Cells were pelleted in a clinical table-top centrifuge. The cells for 4-nitroquinoline-*N*-oxide (NQO), ethylmethanesulfonate (EMS), and methylmethanesulfonate (MMS) treatment were washed once with a 5% solution of sodium thiosulfate to inactivate the agent and a second time with sterile distilled water. For all other chemicals, the cells were washed twice with sterile, distilled water. Thereafter cells were resuspended in 0.5–1 mL of sterile, distilled

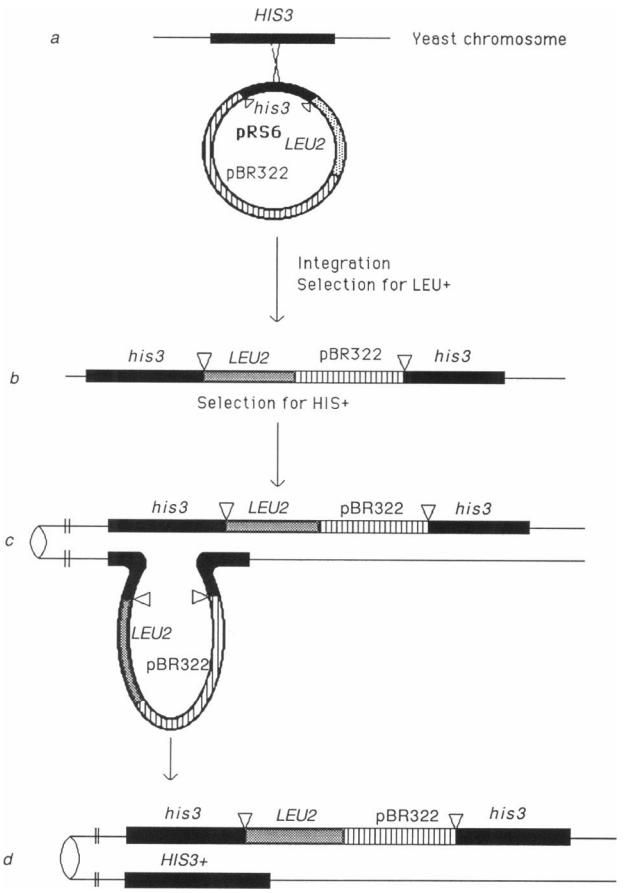

FIGURE 1. Plasmid pRS6, which contains an internal fragment of the *HIS3* gene, was cut within the internal *his3* fragment and integrated into the genome at the *HIS3* locus (*a*). This creates a duplication of the *his3* gene in which one allele is deleted for its 3' end and the other for its 5' end (*b*). The two alleles share about 400 basepairs of homology and thus can recombine with each other to revert to the *HIS3*$^+$ allele. As shown previously, *HIS3*$^+$ recombinants do not arise by plasmid excision or by unequal sister chromatid exchange (*14*). In these studies the frequency of plasmid excision was determined by cloning a yeast origin of replication sequence onto the integrating plasmid. Excised plasmids could be recovered but were found 100 times less frequently than HIS$^+$ formation, suggesting that plasmid excision does not occur in the majority of recombinants. Reciprocal products expected to result from sister chromatid exchange were analyzed by Southern blotting. The pattern characteristic for sister chromatid exchange was not found in any of 25 events examined. Thus, as a likely alternative mechanism, it is suggested that conversion between sister chromatids (*c*) results in deletion of the integrated plasmid on one chromatid (*d*). After segregation of the two chromatids, the HIS$^+$ recombinant should show a HIS$^+$ leu-phenotype, which is found in 99% of all HIS$^+$ recombinants. As a possible model, it is proposed that a double-strand break initiates the recombination event and is then extended by an exonuclease to a gap. This gap is repaired by gene conversion with the sister chromatid acting as donor (*14*).

water, transferred to a glass tube, and sonicated to disperse any clumps. Cells were counted and appropriate numbers were plated onto SC medium to determine the number of survivors, onto SC-HIS medium to score for DEL events, and onto SC-ADE medium to determine the frequency of ICR events. The cells did not contain any clumps of tetrads. Colonies were counted after 2–3 days of incubation at 30°C. All data given reflect the total number of recombination events per number of cells capable of forming colonies (survivors) on nonselective medium.

Data derived from less than five colonies were not included. A minimum increase of two-fold over the spontaneous frequency in a dose-dependent manner was regarded as evidence for inducibility. Each experiment was repeated at least three times, and the results obtained were highly reproducible. Two plates were used for each system and each concentration. Details on irradiation of cells and chemicals used can be found in Table 1.

Results and Discussion

There is evidence in the current literature that substantial genome rearrangements are associated with cancer (*16–22*). Deletions have been implicated in carcinogenesis caused by mutations in recessive oncogenes such as retinoblastoma or colon cancer (*23–24*). Leukemia may be caused by translocations (*25*). Because of the association of genome rearrangement with cancer, a system selecting for intrachromosomal recombination that results in genome rearrangement has been constructed in the yeast *Saccharomyces cerevisiae* [Fig. 1 (*14*)]. A plasmid with an internal fragment of the *HIS3* gene has been integrated at the *HIS3* locus, yielding an integrative disruption of the *HIS3* gene. This resulted in two copies of the *HIS3* gene, each having one terminal deletion. This construct reverts to *HIS3*$^+$ by recombination of the two *his3* deletion alleles. This recombination event deletes 6 kb of DNA that comprise the entire integrated plasmid and has therefore been termed a DEL event [Fig. 1 (15)].

Because of the involvement of deletions in carcinogenesis, the inducibility of DEL recombination with carcinogens has been studied. Carcinogens that are positive in the Salmonella assay (mutagenic carcinogens) as well as carcinogens that are negative in the Salmonella assay (nonmutagenic carcinogens) turned out positive with the DEL assay (*15,26,27*). The yeast strain RS112, containing a duplication of the *HIS3* gene (each carrying different terminal deletions) was used to determine the effects of different carcinogens and other agents on intrachromosomal recombination between the two *his3*$^-$ alleles (DEL recombination). In addition, strain RS112 contained a system to select for ICR between two homologous chromosomes. Carcinogens that are detectable with the Salmonella assay, namely, UV and γ irradiation, MMS, EMS, NQO, nitrogen mustard, epichlorohydrine, aflatoxin B_1, ethylene dibromide, and dimethylhydrazine, induced DEL as well as the ICR frequencies (Table 1).

Carcinogens that are not detectable with the Salmonella assay, namely, formaldehyde, safrole, eugenol, ethionine, urethane, auramine, methylene chloride,

Table 1. Inducibility of intrachromosomal (DEL) and interchromosomal recombination (ICR) by different agents.

Agent	Carcinogen	Response in Salmonella assay	Response in DEL	Response in ICR	Reference
UV irradiation	+	+	+	+	(*27*)
γ-Ray exposure	+	+	+	+	(*27*)
MMS	+	+	+	+	(*15,27*)
EMS	+	+	+	+	(*15,27*)
NQO	+	+	+	+	(*15,27*)
Nitrogen mustard	+	+	+	+	(*15,27*)
Epichlorohydrin	+	+	+	+	(*15,27*)
Aflatoxin B_1	+	+	+	+	(*15,27*)
Ethylene dibromide	+	+	+	+	(*15,27*)
Dimethylhydrazine	+	+	+	+	(*15,27*)
Formaldehyde	+	–	+	+	(*15,27*)
Safrole	+	–	+	+/–	(*15,26,27*)
Eugenol	+	–	+	+	(*26*)
Methyleugenol	?	–	+	+	(*26*)
Ethionine	+	–	+	–	(*15,27*)
Urethane	+	–	+	–	(*15,27*)
Auramine O	+	–	+	–	(*15,27*)
Methylene chloride	+	–	+	–	(*15,27*)
Carbon tetrachloride	+	–	+	–	(*15,27*)
Cadmium chloride	+	–	+	–	(*15,27*)
Cadmium sulfate	+	–	+	–	(*15,27*)
Aniline	+	–	+	–	(*15,27*)
3-Amino-1,2,4-triazole	+	–	+	–	(*15,27*)
Acetamide	+	–	+	–	(*15,27*)
Thioacetamide	+	–	+	–	(*15,27*)
Thiourea	+	–	+	+	(*15,27*)
DDE	+	–	+	+	(*15,27*)
Ethylenethiourea	+	–	+	+	(*15,27*)
Peroxisome proliferators	+	–	–	–	(*28*)
Diethylstilbestrol	+	–	–	–	(*27*)
Mezerein	+	–	–	–	(*27*)
TPA	+	–	–	–	(*27*)
Methionine	–	–	–	–	(*15,27*)
Hydroxylamine HCl	–	–	–	+	(*27*)
Sodium azide	–	+	–	+	(*27*)
5-Bromouracil	–	+	–	–	(*27*)
2-Aminopurine	–	+	+	+	(*27*)
Ethidium bromide	?	+	+	+	(*27*)
4-Aminoantipyrine	?	–	+	+	(*27*)

Abbreviations: MMS; methyl methanesulfonate; EMS, ethyl methanesulfonate; NQO, 4-nitroquinoline-*N*-oxide; dimethylhydrazine, sym-dimethylhydrazine dihydrochloride; DDE, 2,2-bis(chlorophenyl)-1,1-dichloroethylene; peroxisome proliferators, Wy-14,643, methyl clofenopate, nafenopin, ciprofibrate, BR931 and clofibrate were used; ?, equivocal or inconclusive carcinogenicity data.

carbon tetrachloride, cadmium chloride, cadmium sulfate, aniline, 3-aminotriazole, acetamide, thioacetamide, thiourea, DDE, and ethylenethiourea, were all positive with the DEL assay, but most of them did not induce ICR (Table 1). Mutagens that do not show any significant carcinogenic activity (false positives in the Salmonella assay), namely, sodium azide and bromouracil, did not induce DEL recombination but sodium azide induced ICR. On the other hand, aminopurine and ethidium bromide induce both the DEL recombination and ICR.

It may not be surprising that highly tissue-specific carcinogens, carcinogens that may act via hormone imbalance, or tumor promotors would be negative with the yeast system. For instance, peroxisome proliferators, which are liver carcinogens, diethylstilbestrol, tetradecanoyl-13-phorbol acetate and mezerein are negative with the standard DEL assay. Even though six peroxisome proliferators were used (*28*), they were included as one item in the calculation below and in Table 1 because they represent a highly specific group of carcinogens that may act in very similar ways.

The data show that 10 mutagenic carcinogens and 17 carcinogens that are not detectable with the Salmonella assay induced the DEL assay, whereas four carcinogens that are negative with the Salmonella assay were also negative with the DEL assay. The amino acid methionine as well as two noncarcinogenic mutagens (sodium azide, 5-bromouracil) did not induce the DEL assay, whereas the noncarcinogenic mutagen aminopurine induced the DEL system and is therefore a false positive. Thus, in 36 agents there were four false negatives out of 31 carcinogens, which results in a sensitivity of 0.87, and 1 false positive out of 5 noncarcinogens, which results in a specificity of 0.8 and in an overall accuracy of 0.86 as defined in DeSerres and

Ashby (*8*). This means that 86% of the chemicals were identified correctly. Two-thirds of these agents (24 out of 36) have been chosen because they are false negatives or false positives in the Salmonella assay, and thus the Salmonella assay only identified 33% of these agents correctly. This seems to justify the need for further testing to determine the usefulness of the DEL system as a short-term test.

In an international collaborative study that used coded chemicals to evaluate 30 different short-term assays in more than 50 different laboratories (*8*), the carcinogens auramine, safrole, urethane, ethionine, ethylenethiourea, and 3-aminotriazole gave very poor inducibilities with all short-term tests. All of these carcinogens were correctly identified by the DEL assay. When the two recombination systems DEL and ICR were compared in their performance in detecting carcinogens, it was noticed that the mutagenic carcinogens induced both systems well. The nonmutagenic carcinogens, on the other hand, induced DEL recombination much better than ICR. Also, the noncarcinogens hydroxylamine and sodium azide were false positives with the ICR system. Further similarity between carcinogenesis and DEL recombination include that nonmutagenic carcinogens may show a threshold in their dose–response curve, which is not usually seen with mutagenic carcinogens (*29*). Similarly, the nonmutagenic carcinogens formaldehyde, auramine, safrole, carbon tetrachloride, aniline, 3-aminotriazole, acetamide, ethylene thiourea, urethane, and thioacetamide showed a threshold for DEL induction, whereas the mutagenic carcinogens did not show any threshold (*15*).

Characterization of the mechanism involved in the induction of DEL recombination may contribute to the understanding of the biological activity of nonmutagenic carcinogens because induced DEL recombination is the only genotoxic effect some of these agents show. DEL is under different genetic control from ICR and meiotic recombination. Mutations in the DNA repair genes *RAD1*, *RAD10*, and *RAD52* each lower the frequency of DEL recombination about 4-fold (*11,30*). The *RAD52* gene product is involved in double-strand break repair, in ICR and in meiotic recombination (*31*). The *RAD1* and *RAD10* gene products are involved in excision repair and have been shown to have no effect on interchromosomal recombination or meiotic recombination. It has further been shown that the *rad1* and *rad52* mutations act synergistically and reduce DEL recombination to a level far below the effect of each single mutant, suggesting that the *rad1* and *rad52* genes work in different recombination pathways (*11*). These data suggest that the mechanism of DEL recombination differs from that of interchromosomal recombination and meiotic recombination. Furthermore, DEL recombination is UV inducible in the *rad1* mutant but not in the *rad52* mutant (Schiestl, unpublished results). This indicates that the *RAD52* pathway is responsible for the induced recombination. The spontaneous rate is much reduced in the *rad1* mutant. However, induced recombination levels in the *rad1* mutant with UV, EMS, and MMS almost reach wild-type levels so that the *rad1* mutant is more sensitive to detect these agents, especially at lower doses.

Preliminary studies (Schiestl, unpublished observations) defining the phase of the cell cycle in which DEL recombination is inducible have shown that DEL recombination is not inducible in G_1 after UV irradiation, whereas ICR is highly UV inducible in G_1. DEL as well as ICR are not UV inducible in cells synchronized in G_2, whereas both are highly UV inducible in logarithmically growing cells. That ICR is inducible in G_1 but not in G_2 has also been previously shown (*32*). Thus, DEL recombination is UV inducible only in actively growing cells, whereas ICR is in addition highly inducible in G_1. γ-Rays and MMS induce DEL recombination in logarithmically growing and in G_2-arrested cells, but less in G_1-arrested cells. It has also been shown that the nomutagenic carcinogens induced DEL nonmutagenic recombination only in logarithmically growing cells but not in stationary cells (*26*, Schiestl, unpublished observation).

DEL recombination may cycle in its sensitivity for induction during the cell cycle and may be most sensitive in the S and G_2 phases, whereas ICR is most sensitive in G_1. Therefore, DEL recombination may be inducible by agents that damage DNA directly or indirectly in the S phase. For instance, they may interfere with DNA replication or with DNA replication enzymes or with other DNA metabolizing or DNA repair activities. The damage they cause could be repaired in the S or G_2 phase (by sister chromatid recombination) before cell division and therefore they may not induce ICR, which is not inducible in this phase. It is well documented that there is a checkpoint in the G_2 phase of the cell cycle, which is only released when the cellular DNA is intact (*33,34*). That DEL recombination is inducible by the nonmutagenic carcinogens exclusively in proliferating cells may represent another similarity between DEL recombination and carcinogenesis because cell proliferation also seems to be important for carcinogenesis.

Carcinogenesis is a multistage process; multiple genetic events are necessary for neoplastic transformation of a normal cell (*35*). For instance, a mutation of one allele of a tumor-suppressor gene may predispose an individual to cancer but loss of heterozygosity (LOH) of the normal allele has to occur for expression of the transformed phenotype (*23*). LOH may be caused by deletion of the second allele. Provided that DEL events in fact are recombination events between sister chromatids, such events usually do not induce point mutations or ICR and are thus genetically silent. This might explain the nonmutagenic character of carcinogens that only induce DEL. Sister chromatid recombination events may give rise to recombination events between repeated elements, yielding genome rearrangements and bringing proto-oncogenes (*36*)

into proximity with promoter or enhancer sequences. Sister chromatid recombination can also lead to gene amplification, which may also be involved in carcinogenesis (*37*).

If, after further evaluation, results obtained with the DEL assay show high correlation with the carcinogenicity of the test agents, the DEL assay could be useful as a short-term test for predictive carcinogenesis.

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REFERENCES

1. Doll, R., and Peto, R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J. Natl. Cancer Inst. 66: 1192–1308 (1981).
2. Lave, L. B., and Omenn, G. S. Cost-effectiveness of short-term tests for carcinogenicity. Nature 324: 29–34 (1986).
3. Sivak, A., Goyer, M. M., and Ricci, P. F. Nongenotoxic carcinogens: prologue. In: Nongenotoxic Mechanisms in carcinogenesis (B. E. Butterworth and T. J. Slaga, Eds.), Banbury Report 25, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1987, pp. 1–8.
4. McCann, J., Choi, E., Yamasaki, E., and Ames, B. N. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Natl. Acad. Sci. U.S.A. 72: 5135–5139 (1975).
5. Ashby, J., and Tennant, R. W. Definitive relationship among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. Mutat. Res. 275: 229–306 (1991).
6. Butterworth, B. E., and Slaga, T. J., Eds. Nongenotoxic Mechanisms of Carcinogenesis. Banbury Report 25. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1987.
7. Ricci, P. F., and Melton, L. S. Regulating cancer risks. Environ. Sci. Technol. 19: 437 (1985).
8. De Serres, F. J., and Ashby, J. Evaluation of short term tests for carcinogens. Prog. Mutat. Res. 1 (1981).
9. Ashby, J., de Serre, F., Draper, M., Ishidate, Jr., M., Margolin, B. H., Matter, B. E., and Shelby, M. D., Eds. Evaluation of short term tests for carcinogens. Progress in Mutation Research, Vol. 5, Elsevier, Amsterdam, 1985.
10. Tennant, R. W., Margolin, B. H., Shelby, M. D., Zeiger, E., Haseman, J. K., Spalding, J., Caspsry, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor R. Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. Science 236: 933–941 (1987).
11. Schiestl, R. H., and Prakash, S. *RAD1*, an excision repair gene of *Saccharomyces cerevisiae*, is also involved in recombination. Mol. Cell. Biol. 8: 3619–3626 (1988).
12. Fasullo, M. T., and Davis, R. W. Recombinational substrates designed to study recombination between unique and repetitive sequences *in vivo*. Proc. Natl. Acad. Sci. U.S.A. 84: 6215–6219 (1987).
13. Schiestl, R. H., and Wintersberger, U. X-ray enhances mating type switching in heterothallic strains of *Saccharomyces cerevisiae*. Mol. Gen. Genet. 186: 512–517 (1982).
14. Schiestl, R. H., Igarashi, S., and Hastings, P. J. Analysis of the mechanism for reversion of a disrupt d gene. Genetics 119: 237–247 (1988).
15. Schiestl, R. H. Nonmutagenic carcinogens induce intrachromosomal recombination in yeast. Nature 337: 285–288 (1989).
16. Cairns, J. The origin of human cancers. Nature 289: 353–357 (1981).
17. Wintersberger, U. Chemical carcinogenesis – the price for DNA repair? Naturwissenschaften 69: 107–113 (1982).
18. Haluska, F. G, and Tsujimoto, Y., and Croce, C. M. Oncogene activation by chromosome translocation in human malignancy. Annu. Rev. Genet. 21: 321–345 (1987).
19. Varmus, H. E. The molecular genetics of cellular oncogenes. Annu. Rev. Genet. 18: 553–612 (1984).
20 Bishop, J. M. The molecular genetics of cancer. Science 235: 305–311 (1987).
21. Mitelman, F., and Heim, S. Chromosome abnormalities in cancer. Cancer Detect. Prev. 14: 527–537 (1990).
22. Sandberg, A. A. Chromosome abnormalities in human cancer and leukemia. Mutat. Res. 247: 231–240 (1991).
23. Hansen, M. F., and Cavanee, W. K. Tumor suppressors: recessive mutations that lead to cancer. Cell 53: 172–173 (1988).
24. Ponder, B. Gene losses in human tumors. Nature 335: 400–402 (1988).
25. Rowley, J. D. Biological implications of consistent chromosome rearrangements in leukemia and lymphoma. Cancer Res. 44: 3159–3168 (1984).
26. Schiestl, R. H., Chan, W.–S., Gietz, R. D., Mehta, R. D., and Hastings, P. J. Safrole, eugenol and methyleugenol, reputed nonmutagenic carcinogens induce intrachromosomal recombination in yeast. Mutat. Res. 224: 427–436 (1989).
27. Schiestl, R. H., Gietz, R. D., Mehta, R. D., and Hastings, P. J. Carcinogens induce intrachromosomal recombination in yeast. Carcinogenesis 10: 1445–1455 (1989).
28. Schiestl, R. H., and Reddy, J. K. Effect of peroxisome proliferators on intrachromosomal and interchromosomal recombination in yeast. Carcinogenesis 11: 173–176 (1990).
29. Kroes, R. Short term tests in the framework of carcinogen risk assessment to man. Ann. N.Y. Acad. Sci. 407: 398–408 (1983).
30. Schiestl, R. H., and Prakash, S. *RAD10*, an excision repair gene of *Saccharomyces cerevisiae*, is involved in the RAD1 pathway of mitotic recombination. Mol. Cell. Biol. 10: 2485–2491 (1990).
31. Kunz, B., and Haynes, R. Phenomenology of genetic recombination in yeast. Annu. Rev. Genet. 15: 57–89 (1981).
32. Fabre, F., Boulet, A., and Roman, H. Gene conversion at different points in the cell cycle of *Saccharomyces cerevisiae*. Mol. Gen. Genet. 195: 139–143 (1984).
33. Hartwell, L. H., and Weinert, T. Checkpoints: controls that ensure the order of cell cycle events. Science 246: 629–634 (1989).
34. Schiestl, R. H., Reynolds, P., Prakash, S., and Prakash, L. Cloning and sequence analysis of the Saccharomyces cerevisiae *RAD9* gene and further evidence that its product is required for cell cycle arrest induced by DNA damage. Mol. Cell. Biol. 9: 1882–1896 (1989).
35. Weinstein, I. B. The origins of human cancer: molecular mechanisms of carcinogenesis and their implications for cancer prevention and treatment. Twenty-seventh G.H.A. Clowes memorial award lecture. Cancer Res. 48: 4135–4243 (1988).
36. Marx, J. L. The case of the misplaced gene. Science 218: 983–985 (1982).
37. Alitalo, K., and Schwab, M. Oncogene amplification in tumor cells. Adv. Cancer Res. 47: 235–279 (1986).